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Studies on Sialic Acids. V. Synthesis of α- and β-D-Neu5Ac*p*-(2→6)-lactose

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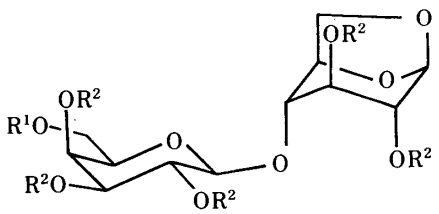
The reaction of methyl 5-acetamide-4,7,8,9-tetra-*O*-acetyl-2-chloro-2,3,5-trideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate with 1,6-anhydro-2,2′,3,3′,4′-penta-*O*-benzyl-β-D-lactose in the presence of mercury cyanide and mercury bromide gave a 1 : 1 mixture of the two anomers of the (2→6) linked trisaccharide. *O*-(Methyl (5-acetamide-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate)-(2→6)-*O*-(2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2,3-di-*O*-benzyl-β-D-glucopyranose and the corresponding β-(2→6) linked isomer could be isolated by chromatography. A deprotection sequence, acetolysis, hydrogenolysis, deacetylation and saponification led to *O*-(5-acetamide-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose and to the corresponding β-(2→6) linked compound.

Keywords——*N*-acetylneuraminic acid; sialosyllactose; NMR; CD; HPLC; sialic acid

Sialosyllactose is one of the simpler, readily available compounds containing *N*-acetylneuraminic acid in a glycoside linkage. Sialosyllactose was first isolated from rat mammary gland by Caputto and Trucco,[1] and later in more substantial yields from bovine colostrum by Kuhn and Brossmer.[2] The two isomers of sialosyllactose were shown to be *O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)-D-glucopyranose (α-D-Neu5Ac*p*-(2→3)-lactose) and *O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-β-D-galactopyranosyl-(1→4)-D-glucopyranose (α-D-Neu5Ac*p*-(2→6)-lactose; **13**) by Schneir and Refelson.[3] As part of a program on the synthesis of glycoconjugates binding sialic acid, we now report the synthesis of α-D-Neu5Ac*p*-(2→6)-lactose (**13**). Recently the synthesis of α-D-Neu5Ac*p*-(2→3)-lactose was reported by Ogawa and Sugimoto.[4]

The starting material, 1,6-anhydro-2,2′,3,3′,4′,6′-hexa-*O*-acetyl-β-D-lactose (**1**), was prepared as described by Tejima[5] and Fujimaki *et al.*[6] *O*-(2,3,4-Tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2,3-di-*O*-benzyl-β-D-glucopyranose (**5**) having all positions protected except 6′-OH was obtained by the following route. The acetyl groups of **1**



1: $R^1=R^2=Ac$ **4**: $R^1=Tr$, $R^2=Bn$
2: $R^1=R^2=H$ **5**: $R^1=H$, $R^2=Bn$
3: $R^1=Tr$, $R^2=H$ **6**: $R^1=Ac$, $R^2=Bn$
Ac=acetyl, Bn=benzyl, Tr=trityl

Chart 1

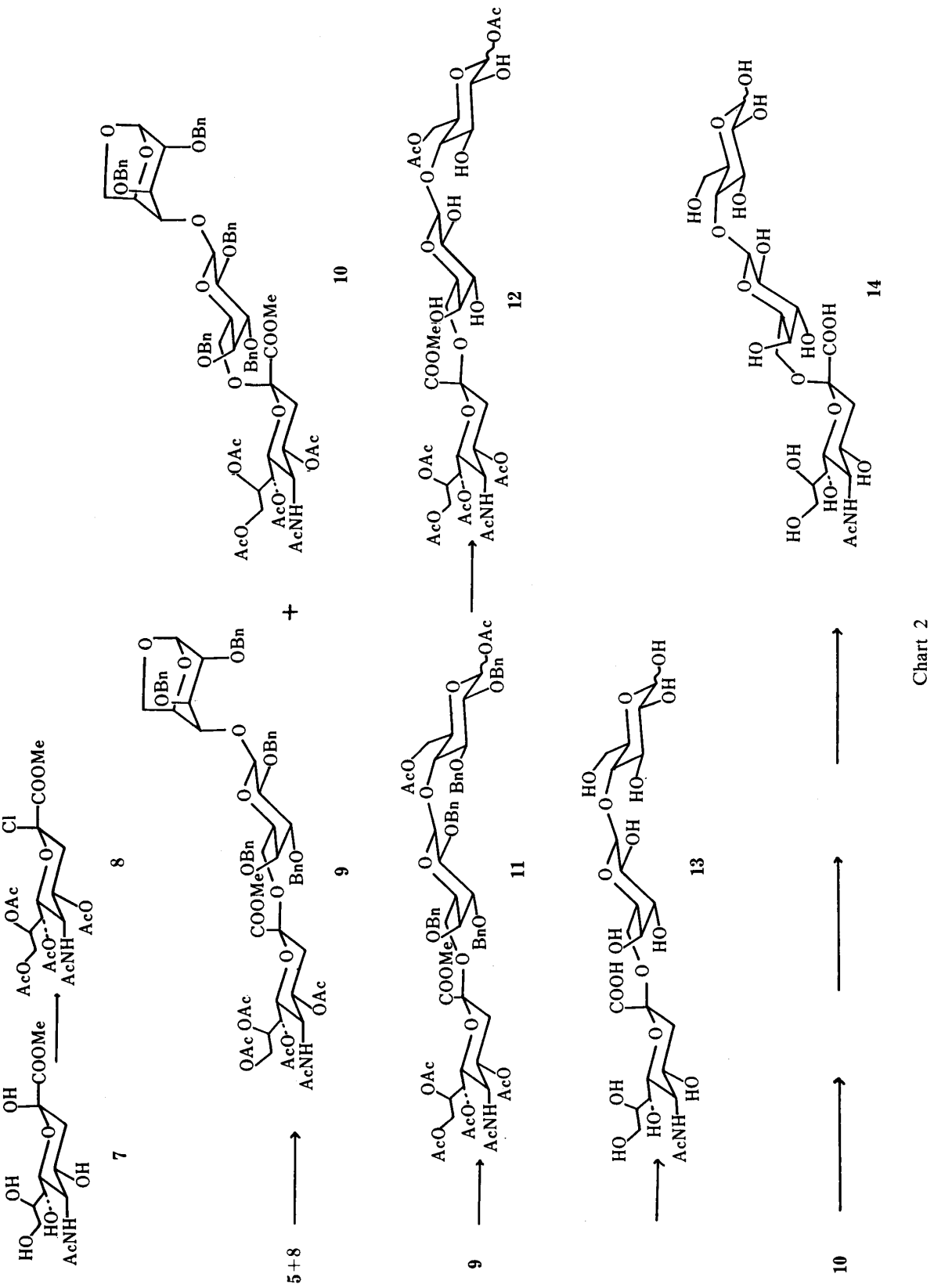


Chart 2

were removed by treatment with 0.1 M sodium methoxide to give **2**. This product was tritylated, and then benzylated to give the fully protected intermediate **4**. Compound **4** was detritylated to give crystalline **5** in 30% overall yield from **1**.[7] Acetylation of 5 gave 6′-*O*-acetyl-1,6-anhydro-2,2′,3,3′,4′-penta-*O*-benzyl-β-D-lactose (**6**). Comparison of the carbon chemical shifts of C-6′ in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra (in $CDCl_3$) of **5** and **6** showed that acetylation of position 6′ of **5** shifted the signal to δ 61.9 ppm from δ 63.3 ppm in **6**.

On the other hand, we have found a facile method for the preparation of methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-chloro-2,3,5-trideoxy-D-*glycero*-β-D-*glacto*-2-nonulopyranosonate (**8**) by treatment of methyl (5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate) (**7**) with an excess amount of acetyl chloride at room temperature. Compound **8** was obtained as fine crystals; **8** has previously been prepared as a syrup by Kuhn *et al.*[8]

Condensation of **5** and **8** in dry dichloromethane in the presence of mercury cyanide and mercury bromide gave a mixture of four major components as determined by thin layer chromatography (TLC). The reaction mixture was separated by column chromatography on silica gel to give *O*-(methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate)-(2→6)-*O*-(2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2,3-di-*O*-benzyl-β-D-glucopyranose (**9**) in 21% yield and the corresponding β-(2→6) linked compound (**10**) in 8% yield. From the proton nuclear magnetic resonance (^{1}H-NMR) spectra and field desorption-mass spectra (FD-MS), **9** and **10** were confirmed to be the protected sialosyllactose compounds. In this case, the by-product methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enonate was formed in about 30% yield.[9,10]

In the deprotection of **9**, the 1,6-anhydro-β-ring in **9** was converted into acetyl groups by treatment with acetic anhydride and trifluoroacetic acid to obtain the intermediate **11**. The benzyl groups in crude **11** were removed by catalytic hydrogenation over palladium-on-charcoal and the product was next chromatographed on a column of silica gel. The major eluate fractions contained an α and β anomeric mixture of the trisaccharide **12**. The content was estimated from the ratio of C-1 anomeric proton signals in the ^{1}H-NMR spectrum of **12**. Deacetylation and saponification of **12** with aqueous sodium hydroxide afforded α-D-Neu5Ac*p*-(2→6)-lactose (**13**) as an amorphous powder in 7.8% overall yield from **7**. β-D-NeuAc*p*-(2→6)-lactose (**14**) was obtained by acetolysis, catalytic hydrogenolysis and saponification of **10** in 3.8% overall yield from **7** as described for **13**.

The anomeric configuration of *N*-acetylneuraminic acid derivatives can usually be inferred from the chemical shift of 3″-H (eq).[11] The ^{1}H-NMR spectra showed the signal due to 3″-H (eq) at δ 2.70 for **13** and δ 2.43 for **14**, indicating α and β configuration, respectively.

The three anomeric carbon atoms of the monosaccharide components of **13** and **14** were assigned from the ^{13}C-NMR spectra, as shown in Table I.[12,13] In this case, comparison of the chemical shifts at the anomeric carbon atom (C-2″) in the *N*-acetylneuraminic acid moiety of **13** and **14** showed that the signal of the β-anomeric carbon atom is shifted downfield from that

Table I. ^{13}C-NMR Signals (ppm) of the Anomeric Carbon Atoms in **13**, **14**, and α-D-Neu5Ac*p*-(2→3)-lactose[13]

Carbon number	**13**	**14**	α-D-Neu5Ac*p*-(2→3)-lactose
Glc (1α)	92.7	92.7	92.0
Glc (1β)	96.6	96.7	95.9
Neu5Ac (2″)	96.7	101.2	100.0
Gal (1′β)	103.8	104.0	102.8

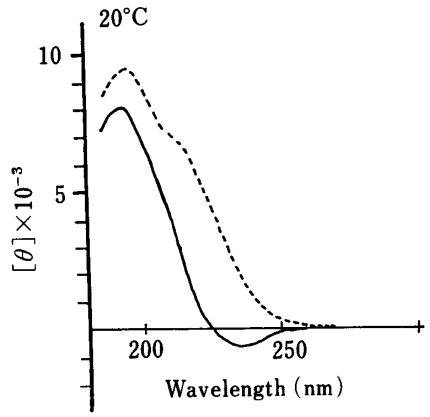


Fig. 1. CD Curves of **13** (——) and **14** (------) in H_2O

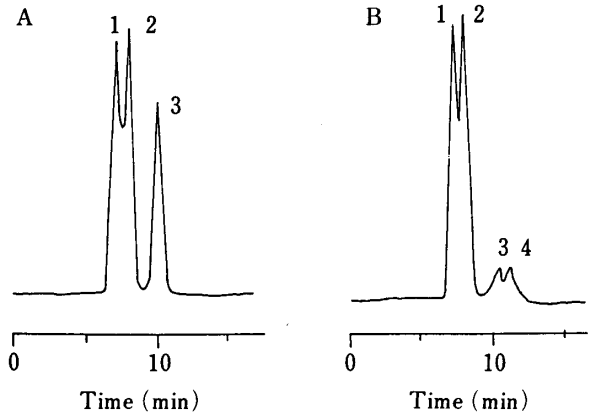


Fig. 2. HPLC Separation of a Mixture of **13** (Peak 1), **14** (Peak 2), *N*-Acetylneuraminic Acid (Peak 3), and Lactose (Peak 4)

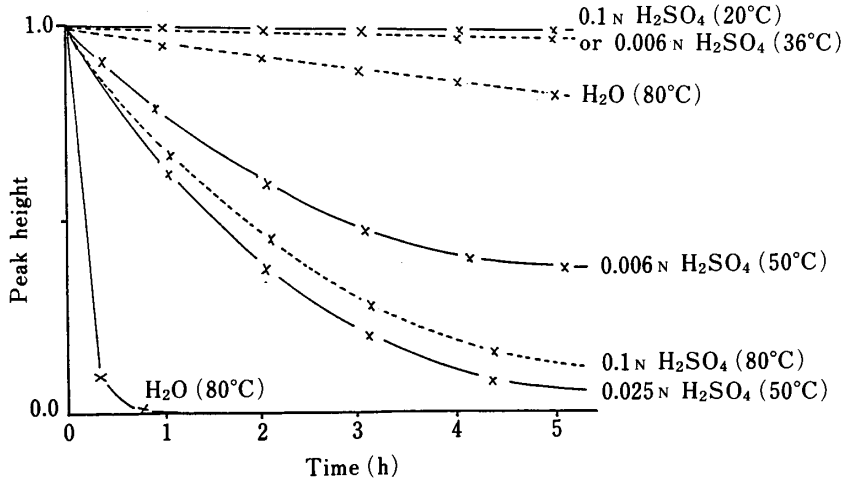


Fig. 3. Hydrolysis of **13** (——) and **14** (------)

of the α-anomer.

Figure 1 shows the circular dichroism (CD) spectra of **13** and **14**. The stereochemistry of the synthetic product **13** was identified by comparison of its CD curve with that of a natural sample.[14-16]

An ion-exchange, high-performance liquid chromatography (HPLC) method has been developed to separate *N*-acetylneuraminic acid and sialosyl oligosaccharides.[17,18] Application of this method to the analysis of the sialosyllactose and lactose was examined, using an Aminex HPX-87 strong cation exchange resin column, and it was found that this method afforded high resolution of *N*-acetylneuraminic acid, **13**, **14**, and lactose, as shown in Fig. 2. The synthetic **13** and the natural product, α-D-Neu5Ac*p*-(2→6)-lactose, were shown to be identical by using this HPLC method. Further confirmation of the configuration at the anomeric position of the *N*-acetylneuraminic acid moiety was provided by measurement of the rate of hydrolysis.[19] Figure 3 shows the rates of hydrolysis of **13** and **14** under various conditions based on the peak height ratio in HPLC. When the hydrolysis proceeded at 80 °C, the α-anomer (**13**) was rapidly hydrolyzed to *N*-acetylneuraminic acid and lactose, while the β-amoner (**14**) was hydrolyzed very slowly.

In conclusion, it is clear that measurement of the rate of hydrolysis can be an important method for the confirmation of anomeric configuration.

Experimental

General Methods——The ^{1}H-NMR spectra were measured with Varian T-60 or Varian EM-390 spectrometer, and the ^{13}C-NMR spectra were obtained at 25 MHz in the pulsed Fourier-transform mode on JEOL FX-100 instruments with Me_4Si (TMS) as an internal standard for $CDCl_3$ and $Me_3Si(CH_2)_3SO_3Na$ (DSS) for D_2O at 32 °C. The FD-MS were obtained on a JEOL JMS-DX 300, infrared (IR) spectra on a JASCO IR-A2 spectrometer, and CD spectra on a JASCO J-20 spectrometer in 1 and 0.2 mm cells (the concentration and cell length were adjusted to obtain the maximum signal). Optical rotations were measured with a JASCO-JIP-4 digital polarimeter. TLC was conducted on precoated silica gel plates (Merck GF-254), and the detection of compounds was achieved by UV fluorescence quenching and with 5% sulfuric acid solution.

Materials——*N*-Acetylneuraminic acid was isolated under mild aqueous hydrolytic conditions from edible bird's nest substance (purchased from a Chinese restaurant) in high yield and without contamination with other acylneuraminic acids by utilizing a modification of the procedure of Czarnicki and Thornton.[13] Methyl 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranonate (**7**) was prepared according to the method of Kuhn *et al.*[8] Penta-*O*-acetyl-1,6-anhydrolactose (**1**) was obtained by the procedure of Tejima.[5] Natural α-D-Neu5Ac*p*-(2→6)-lactose was isolated from bovine colostrum using the ion-exchange procedure of Schneir and Rafelson.[3]

***O*-(2,3,4-Tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2,3-di-*O*-benzyl-β-D-glucopyranose (5)**——A 0.1 M sodium methoxide solution (10 ml) was added to a suspension of 1,6-anhydro-2,2′,3,3′,4′,6′-hexa-*O*-acetyl-β-D-lactose (**1**, 10 g) in dry methanol (150 ml), and the mixture was kept for 3 h at room temperature. The solution was neutralized with Dowex-50 (H^+) ion-exchange resin. The resin was filtered off, and the filtrate was evaporated and dried over P_2O_5 to give **2** as an amorphous material. A mixture of **2** and trityl chloride (7 g) in dry pyridine (200 ml) was stirred at room temperature with exclusion of moisture. After 16 h, a further portion of trityl chloride (4 g) was added, and the stirring was continued for 4 h at 60 °C. The pyridine solution was added dropwise to ice water, and the mixture was extracted with chloroform (2 × 100 ml). The combined extracts were successively washed with ice-water, ice-cold 1 N HCl, 3% $NaHCO_3$, and water, and filtered through Celite. The clear solution was diluted with an equal volume of petroleum ether and the precipitate of the crude trityl ether (**3**) was collected by filtration. Sodium hydride (8 g) was added in portions to a stirred solution of **3** in dimethylformamide (100 ml) and benzyl chloride (30 g), and stirring was continued for 12 h at room temperature. The excess of sodium hydride was decomposed by the addition of methanol and water, and the solvents were evaporated off. The product was extracted with ethyl acetate, and the extract was washed with ice-cold 1 N hydrochloric acid and saturated sodium chloride solution, dried over sodium sulfate, and evaporated to give the benzyl ether (**4**). The product, **4** was treated with 1 N hydrochloric acid–acetone (1: 10, 100 ml) at reflux for 2 h at 90 °C. TLC in ether showed the presence of the alcohol (**5**, *Rf* 0.11) and triphenylmethanol (*Rf* 0.9). The acid was neutralized with an excess of sodium hydrogen carbonate and the solvent was evaporated off. The residue was extracted with ether and the extract was dried over potassium carbonate then evaporated. The residue oil was chromatographed on silica gel (Merck Kieselgel 60) and the product was eluted with ether–light petroleum (1 : 1). It crystallized slowly on standing and was recrystallized from ether–ethyl acetate (9 : 1) to yield 4.0 g (30%) of **5** as colorless needles. mp 119—120 °C, $[\alpha]_D^{26}$ +3.0° (*c* = 1, MeOH). *Anal.* Calcd for $C_{47}H_{50}O_{10}$: C, 72.87; H, 6.46. Found: C, 72.65; H, 6.67. IR ν_{max}^{KBr}: 3500 cm^{-1}. ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.75 (1H, br s, 6′-OH), 5.54 (1H, br s, 1-H), 7.2—7.5 (25H, Ph × 5). ^{13}C-NMR ($CDCl_3$, TMS) δ: 102.7 (C-1), 101.1 (C-1′), 65.5 (C-6), 61.9 (C-6′).

6′-*O*-Acetyl-1,6-anhydro-2,2′,3,3′,4′-penta-*O*-benzyl-β-D-lactose (6)——A solution of **5** (100 ml) in dry pyridine (1 ml) containing acetic anhydride (1 ml) was kept for 6 h at room temperature. The reaction solution was poured into water and extracted with chloroform. The extract was washed with water, dried and concentrated to give **6** (920 mg) as an amorphous material. $[\alpha]_D^{26}$ +2.6° (*c* = 1, MeOH). *Anal.* Calcd for $C_{49}H_{52}O_{11}$: C, 72.04; H, 6.42. Found: C, 71.95; H, 6.62. IR ν_{max}^{KBr}: 1740 cm^{-1} (-OAc). ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.87 (3H, s, -OAc), 5.41 (1H, br s, 1-H), 7.2—7.5 (25H, Ph × 5). ^{13}C-NMR ($CDCl_3$, TMS) δ: 103.1 (C-1), 101.2 (C-1′), 65.3 (C-6), 63.3 (C-6′).

Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2-chloro-2,3,5-trideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate (8)——A suspension of **7** (1 g) in acetyl chloride (30 ml) was stirred for 16 h at 35 °C. The reaction mixture was evaporated to a syrup. This was dissolved in dry benzene and the solution was evaporated to dryness at 30 °C. This was repeated twice, and the residue was crystallized from ether–hexane to give **8** (1.26 g, 80%). mp 116—118 °C, $[\alpha]_D^{20}$ −68° (*c* = 1, $CHCl_3$). *Anal.* Calcd for $C_{20}H_{28}ClNO_{12}$: C, 47.11; H, 5.53; N, 2.75. Found: C, 47.09; H, 5.56; N, 2.74. IR ν_{max}^{KBr}: 1735, 1654, 1532 cm^{-1}. ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.92 (3H, s, NAc), 1.98—2.10 (12H, OAc × 4), 2.78 (1H, dd, *J* = 5.0, 12.0 Hz, 3-H_{eq}), 3.91 (3H, s, COOMe).

***O*-[Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2→6)-*O*-(2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2,3-di-*O*-benzyl-β-D-glucopyranose (9) and *O*-[Methyl(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranoxyl)onate]-(2→6)-*O*-(2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-1,6-anhydro-2,3-di-*O*-benzyl-β-D-glucopyranose (10)**——A solution of **5** (30 g) and **8** (15 g) in dry dichloromethane (300 ml) was stirred with mercury cyanide (10 g) and mercury bromide (5 g) for 60 h at room temperature. The reaction solution was evaporated to dryness at 40 °C under reduced pressure. The residue was dissolved in ethyl acetate (300 ml) and the solution was washed with 30% potassium iodide aqueous solution to remove mercury cyanide and mercury bromide. The ethyl acetate solution was

dried over sodium sulfate and filtered, and the filtrate was evaporated to dryness. The residue was taken up in ether (100 ml), the suspension kept overnight, and the deposited, crystalline **5** was filtered off. The filtrate was evaporated to a syrup, which was chromatographed on a column of silica gel (Merck, Kieselgel 60) with ethyl acetate–benzene. The first fraction contained the unreacted starting material (**5**). The succeeding fractions gave an amorphous by-product (8.3 g, 60%) which was identified as an elimination product, methyl 4,7,8,9-tetra-*O*-acetyl-2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid, derived from **8** on the basis of TLC, elemental analysis, ^{1}H-NMR spectra and optical rotation.[9] Fractions having *Rf* 0.30 in TLC in 1 : 1 (v/v) benzene–ethyl acetate were combined and evaporated to give **9** (8 g, 21%) as a white powder. $[\alpha]_D^{26}$ −30.2° (*c* = 1, $CHCl_3$). FD-MS *m/z*: 1248 ($M^+ + 1$), 1270 ($M^+ + 23$(Na)). *Anal.* Calcd for $C_{67}H_{77}NO_{22}$: C, 64.46; H, 6.21; N, 1.12. Found: C, 64.32; H, 6.05; N, 1.11. IR ν_{max}^{KBr}: 1740, 1675, 1530 cm^{-1}. ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.8—2.1 (16H, OAc, NAc, and 3″-H_{ax}), 2.53 (1H, dd, *J* = 3.5, 13.5 Hz, 3″-H_{eq}), 3.58 (3H, s, COOMe), 5.43 (1H, br s, 1-H), 7.1—7.5 (25H, Ph × 5).

Fractions having *Rf* 0.26 in TLC in 1 : 1 (v/v) benzene–ethyl acetate were combined and evaporated to give **10** (3 g, 8%) as a white powder. $[\alpha]_D^{26}$ −27.0° (*c* = 1, $CHCl_3$). FD-MS *m/z*: 1248 ($M^+ + 1$), 1270 ($M^+ + 23$(Na)). *Anal.* Calcd for $C_{67}H_{77}NO_{22}$: C, 64.46; H, 6.21; N, 1.12. Found: C, 64.46; H, 6.35; N, 1.08. IR ν_{max}^{KBr}: 1740, 1675, 1530 cm^{-1}. ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.70 (3H, s, NAc), 1.90, 1.94, 2.08, and 2.09 (OAc × 4), 2.25 (1H, dd, *J* = 3.8, 13.0 Hz, 3″-H_{eq}), 3.66 (3H, s, COOMe), 5.40 (1H, br s, 1-H), 7.1—7.5 (25H, Ph × 5).

***O*-[Methyl (5-acetamido-4,7,8,9- tetra-*O*-acetyl- 3,5-dideoxy-D-*glycero*-α-D-*galacto*-2- nonulopyranosyl)onate]-(2→6)-*O*-(β-D-galactopyranosyl)-(1→4)-di-*O*-acetyl-D-glucopyranose (12)**——A mixture of acetic anhydride (10 ml) and trifluoroacetic acid (0.8 ml) was cooled to 0 °C and added to **9** (1.0 g). This solution was kept for 8 h at room temperature, then poured into sodium hydrogen carbonate in water under stirring at 0 °C, and extracted with ethyl acetate (2 × 50 ml). The extract was washed with saturated sodium chloride solution, dried, and evaporated. The crude product **11** was obtained as an amorphous powder. A solution of the crude **11** in glacial acetic acid (20 ml) was treated with hydrogen over 5% palladium-on-charcoal for 12 h at room temperature. The solution was filtered through Celite and evaporated to a syrup, which was chromatographed on silica gel with ethyl acetate–ethanol. The eluate showing a spot at *Rf* 0.40 in TLC in chloroform–ethanol (9 : 1, v/v) was evaporated to dryness to give pure **12** (430 mg, 60%) as an amorphous powder. $[\alpha]_D^{22}$ +20° (*c* = 1, MeOH). *Anal.* Calcd for $C_{36}H_{53}NO_{25}$: C, 48.05; H, 5.93; N, 1.55. Found: C, 47.98; H, 5.92; N, 1.51. IR ν_{max}^{KBr}: 3400, 1730, 1660, 1540 cm^{-1}. ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.86 (3H, s, NAc), 2.00—2.20 (18H, OAc × 6), 2.60 (1H, dd, *J* = 13.0, 13.5 Hz, 3″-H_{eq}), 3.78 (3H, s, COOMe), 5.50 (1/3H, d, *J* = 8.0 Hz, 1-H_{eq}), 6.15 (2/3H, d, *J* = 2.7 Hz, 1-H_{ax}).

***O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose [α-D-Neu5Ac*p*-(2→6)-lactose, 13]**——A solution of **12** (430 mg) in 1 N sodium hydroxide (2 ml) was stirred for 2 h at room temperature. This solution was diluted with water (10 ml), and de-ionized on Dowex-50 (H^+) resin at 0 °C. The filtrate was treated with a small amount of activated charcoal, then filtered through Celite, and the filtrate was evaporated to dryness at 20 °C. The residue was dissolved in a little water, and precipitated with ethanol to give **13** (210 mg, 77%) as an amorphous powder. $[\alpha]_D^{27}$ +5.6° (*c* = 1, H_2O). *Anal.* Calcd for $C_{23}H_{39}NO_{19}$: C, 43.60; H, 6.20; N, 2.21. Found: C, 43.54; H, 6.22; N, 2.28. IR ν_{max}^{KBr}: 3400, 1728, 1660, 1543 cm^{-1}. ^{1}H-NMR (D_2O, DSS) δ: 1.74 (1H, dd, *J* = 12.0, 11.4 Hz, 3″-H_{ax}), 2.0 (3H, s, NAc), 2.70 (1H, dd, *J* = 12.0, 3.6 Hz, 3″-H_{eq}), 4.40 (1H, d, *J* = 6.6 Hz, 1″-H), 5.20 (1/3H, d, *J* = 3.6 Hz, 1-H_{eq}), 4.40 (2/3H, d, *J* = 8.5 Hz, 1-H_{ax}). CD (H_2O) $[\theta]_{232}^{27}$ −520, $[\theta]_{197}^{27}$ +8230.

***O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose [β-D-Neu5Ac*p*-(2→6)-lactose, 14]**——**10** (1 g) was deprotected and purified as described for **13**, to give **14** (300 mg, 60%) as an amorphous powder. $[\alpha]_D^{27}$ +1.8° (*c* = 1, H_2O). *Anal.* Calcd for $C_{23}H_{39}NO_{19}$: C, 43.60; H, 6.20; N, 2.21. Found: C, 42.98; H, 6.53; N, 2.10. IR ν_{max}^{KBr}: 3400, 1728, 1660, 1550 cm^{-1}. ^{1}H-NMR (D_2O, DSS) δ: 1.63 (1H, dd, *J* = 12.60, 11.4 Hz, 3″-H_{ax}), 2.03 (3H, s, NAc), 2.43 (1H, dd, *J* = 12.6, 4.2 Hz, 3″-H_{eq}), 4.41 (1H, d, *J* = 6.6 Hz, 1′-H), 5.20 (1/3H, d, *J* = 3.6 Hz, 1-H_{eq}), 4.41 (2/3H, d, *J* = 8.5 Hz, 1-H_{ax}). CD (H_2O) $[\theta]_{199}^{27}$ +9713.

The Hydrolysis of 13 and 14——**13** and **14** were quantitated by HPLC after hydrolysis at a molar concentration of 4 mM (0.5 mg/0.2 mol) under various conditions, as shown in Fig. 3.

HPLC Methods——α-D-Neu5Ac*p*-(2→6)-lactose (**13**) and α-D-Neu5Ac*p*-(2→6)-lactose (**14**) were analyzed by cation exclusion chromatography using an Aminex HPX-87H strong cation exchange resin column designed for organic acid analysis, (300 × 7.8 mm) at 50 °C (Bio-Rad Laboratories, Richmond, CA, U.S.A.). A mobile phase of 0.006 N sulfuric acid was used at a flow-rate of 0.66 ml/min. The column effluent was monitored with a UV detector at 206 nm (Nihon Seimitsu Kagaku, model NS-310) and an RI detector (Japan Analytical Industry, model RI-2).

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